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HUMAN IMMUNE RESPONSES DURING SCHISTOSOMIASIS MANSONI

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Studies of immune responses as they occur in patients with schistosomiasis appear to progress relative to corrent technological advances, and to advance despite the understandable inability to pursue in vivo *manipulations in this host/parasite system. Emphasis is most often placed on making immunological comparisons between such patient groups as reinfected/non-reinfected, intestinals/hepatosplenic, high/low intensities of infection, infected/uninfected within endemic areas, and those born of infected/uninfected mothers. Based on these types of comparisons, reasonable conjectures can be made regarding the immunological occurrences during this chronic exposure condition. Some consideration is now being given to the immune mechanisms of some of the observations made, and while some of these must then be carried back to experimental models for further manipulation-based analysis, new technological developments continue to assist in the field/bench ability to ask questions that might assist our understanding to a point where this knowledge can be applied to shaping developmental approaches to vaccine development and the goal of alleviating morbidity.*

Key-words: Schistosomiasis. Immune responses. Immunoregulation.

The immune systems of humans infected with *Schistosoma mansoni* are exposed to a wide variety of complex, parasite-derived antigenic substances, and are known to respond with multiple immune mechanisms. Reasonable progress has been made by several groups of investigators in the cataloging of these responses and their regulation. The results of such studies are usually interpreted in regard to one of two very different contests a) resistance to reinfection after chemotherapy; b) morbidity as seen in the differential clinical forms of infection, or related to fecal egg counts (intensity of infection) (1-3). This working paper will summarize these immunological findings and then outline how these findings are currently, or might be, interpreted.

Responses of peripheral blood mononuclear cells to schistosome antigens

A few human immunology studies in schistosomiasis have been done using cells from the spleens of hepatosplenic patients removed at surgery, but by far most studies have analysed the reactivities of peripheral blood mononuclear cells (PBMC). It must be remembered, therefore, that while is the only logically available window into the immune system, it suffers by only a glimpse of what might be occuring in the peripheral lymphoid organs, or even more critically, at the sites of resistance effector involvement or lesion formation. Nevertheless, PBMC have been studied in multiple ways, involving their proliferative capacity, their ability to interact with antigen-containing nidi, and their abilities to make and realease cytokines.

Proliferation

The most commonly reported assay is the proliferative response of PBMC in the presence of schistosome antigenic materials or phytomitogens. Several groups have followed these responses to different schistosome-derived materials [6 14 26 32 58 62]. Most commonly, crude extracts of eggs (SEA), adult worms (SWAP or adult Ag), or cercariae (CERC or CAP) have been used, and have surprisingly provided reather distinct profiles of responses in different types of patients[12]. This is surprising because of the considerable overlap of the antigenic mosaics presented in these sorts of extracts, yet there seem to

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be some dominant components which govern the overall responsiveness to be done to dissect these responses in regard to individual antigenic components within such extracts[5] (B Doughty: personal communication).

Until very recently all such studies were assayed only by the level of incorporation of ^{3}H-TdR into the DNA of those cells stimulated to proliferate in these assay. Current studies also include phenotypic studies of the responding blast cells by flow cytometric analysis[59]. These preliminary studies indicate that the cells respondig to SEA, id and the "super-antigen" staphylococcal enterotoxin B do so in different proportions, as phenotypically characterized by their CD markers, T cell receptor and interleukin receptor expression. Further analysis and correlations with functional attributes assigned to different phenotypes may help in an understanding of the interactions in these responses.

While true longitudinal studies are few[12 14 57], in general, it is clear that patients with early infections respond strongly to SEA, while response to SWAP and CERC develop more slowly[34 53]. As infection progresses into the more chronic phases, a general pattern is seen wich leads to lower anti-SEA proliferative responses in the face of higher responses to SWAP and variable anti-CERC responsiveness. Also, if infections are cured and the former patients are not re-exposed to the threat of schistosome infection their PBMC express very high levels of anti-SEA proliferation[15 35]. Furthermore, it has recently been seen that those individuals who live in endemic areas and have continued water contact , but are repeatedly stool-negative (who are presumed to have self-cured or be putatively resistant; antigenic extracts and purified moieties[5] (G Gazzinelli: data not published).

Numerous different correlations of high or low proliferative responses to various extracts have been made with clinical stages and forms of the infection. Some correlations are seen only in areas of high intensity infections[12 15], while not being observed in populations with moderate or low numbers of egg per gram (epg) of feces. With the emergence or more studies, it is become apparent that both the intensity and the prevalence of a given area may influence or shape the general responsiveness of the population under study (G Gazzinelli: work in progress).

In vitro *granuloma formation*

The immunopathology of experimental *S. mansoni* infection is generally attributed to the granuloma formation around tissue-deposited eggs, and is considered to be a T cell-mediated immune response. Except for the work of Rocklin et al[62], who observed a correlation between suppression of SEA-stimulated cultures and smaller rectal egg-induced granulomas, most information about granuloma formation/modulation in humans has been obtained by examining infeced patients' PBMC reactivity to antigen-conjugated polyacrylamide beads in a so-called, *in vitro*, granuloma assay[23]. These studies suggest that immunoregulation in chronic schistosomiasis is predominantly cellular in nature, particulaly implicating a CD8+ T lymphocyte in the regulation of this reactivity. In addition it has benn reported that anti-idiotypic (anti-Id) CD4+ and CD8+ T cell-subsets can down-regulate autologous granuloma formation *in vitro* when previously incubated with either an anti-SEA human Ig mAb or polyclonal anti-SEA antibodies immunoaffinity-purified from a pool of sera from chronically infected patients. These antibodies suppress the autologous granuloma formation by interaction with anti-Id T cells. This effect was not observed with Fab fragments of anti-SEA antibiodies, suggesting crosslinking of T cell membrane components is required to induce this phenomenon, as it is for the induction of anti-Id T cell proliferation[23]. Specific and non-specific humoral factors can also modulate the immune reponse at the *in vitro* granuloma level[36]. When treated with sera from chronic schistosomiasis patients, PBMC from patients with active schistosome infections induced inhibition of *in vitro* granuloma formation. Significant modulation also occurred upon treatment of PBMC with isolated immune complexes (IC), or with manufactured IC of SEA and purified IgG from pooled chronic schistosomiasis sera. In contrast, the incubation of PBMC with F $(ab')_2$ fragment IgG-SEA IC did not induce any suppression of the granulomatous reactivity to SEA. Addition of indomethacin to the granuloma culture significantly reduced *in vitro* granuloma modulation. Circulating IC may regulate granulomatous hypersensitivity to *S. mansoni* eggs in patients with chronic intestinal schistosomiasis by inducing macrophages to

secrete suppressive prostaglandins[36].

Cytokine production

Little information is available on cytokine production by PBMC of schistosomal patients, relative to the number of PBMC proliferative studies. Fortunately, the last few yers has begun to see this lack now beginning to be rectified by several groups of investigators. Feldmeier and colleagues [29 31] reported a reversal of suppession of concanavalin A stimulation of PBMC from patients with high intensities of infection by exogenous IL-2. Their further studies indicate that a deficit of both IFN-gamma and IL-2 production after both concanavalin A and SEA exposure is reversed upon chemotherapy within 3 months [intestinal (INT) patients] or 6 months [hepatosplenic (HS) patients] after treatment[67]. Bahia-Oliveira et al[5] have stated that SWAP-induced production of IFN-gamma is very low by Int patients' PBMC, but PBMC of endemic normals respond to SWAP exposure by reasonable production of IFN-gamma. It was found the same situation, with strong IFN-gamma production by PBMC of endemic normals, but not those of INT patients, upon exposure to SEA (IRC Viana: personal communication, 1992). In these examples the proliferative and cytokine production responses occur in parallel. Wilson et al.[66] noted that PBMC from schistosomiasis patients generally yielded higher levels of IL-1 than those from uninfected controls, with greater levels produced by those with high intensities of infection. A subgroup of patients with strong adherent cell suppression produced lower levels of IL-1 than did other patients. Earlier studies reported production of Leukocyte Inhibition Factor and Mitogenic Factor[33 46] and neutrophil Chemotaxis Factor[21] by either PBMC of former patients, or patients' splenocytes.

Current immunological evidence, in both murine and human systems, links lymphocyte functions closely with their production and/or usage of given cytokines[52]. Thus it is clear that further knowledge of the cytokine repertoire used by patients PBMC in different settings and to different antigens is desirable. With the advent of the appropriate antibodies for cytokine detection[61], and the molecular capabylities of demonstrating transcription of specific cytokine mRNAs[39] in murine schistosomiasis, the opportunities for studies on this important topic are beginning to unfold.

Responses of PBMC to idiotypes on anti-schistosome antibodies

Investigations in both experimental and clinical schistosomiasis demonstrate the occurence of idiotypic/anti-idiotypic (Id/a-Id) interactions, and some interesting correlations with immunoregulation, morbidity and resistance[17]. Because of the schistosomiasis age-prevalence curves documented from many endemic areas, where the paks of prevalence and intensity are often between 15 and 35 years of age, it is apparent that many children living in endemic areas must have been born of actively infected mothers. Thus the developing immune systems of these children were exposed in utero to the possibilities of manipulation by schistosomal antigens, some of which might cross the placenta or appear in mother's milk, and maternal anti-schistosomal antibodies which may express regulatory idiotypes, capable of shaping and influencing the subsequent expression of the child's immune repertoire. There is evidence that such influences exist[27], and that cord blood mononuclear cells (CBMC) of children born to infected mothers often respond to schistosomal antigen (Novato-Silva et al: submitted for publication) and to the Ids on their mother's anti-SEA antibodies, and those of other chronic INT patients[17 27].

The Id/a-Id responses of PBMC were observed in a proliferative system whereby patients' (or in the above case, neonates born to patients) lymphoid cells respond upon exposure to immunoaffinity-purified anti-SEA antibodies (or anti-SEA mAbs) which express definable, dominant, cross-reactive Ids[49 50]. These Ids stimulate anti-Id T cells, and do so in a direct (non-processed, non-HLA-presented) manner[60]. These stimulatory Ids are found on anti-SEA antibodies from Int patients, but not those with HS disease[51], as will be discussed in another section. This stimulation is specific, and the Ids on these antibodies can be defined serologically with specific rabbit anti-Id sera or mouse anti-Id mAbs[50 51]. The consequences of being a human born of an infected mother and having an already, schistosome-related, manipulated immune system are unknown. Preliminary data on mouse studies of neonatal Id manipulations indicated that whether the Id or anti-

Id is administered at birth can alter the longevity of the mouse upon subsequent infection 8 weeks later. Those receiving a stimulatory anti-SEA mAb that expresses a cross-reactive Id lived longer with their infection than those which received comparable injections of the anti-Id (SM Eloi-Santos: personal communications). Other human studies with these Id/a-Id interactions are also being followed in Kenya, where the opportunities exist to scrutinize the system in migrant populations where some children born of uninfected mothers are raised in endemic areas[8]. Regardless of whether the apparently dramatic findings in neonatal mice occur in humans, the likelihood that many children born in endemic areas may express different immune repertoires than non-endemic individuals, should be taken into consideration, in regard to both morbidity studies and vaccine development.

Antibody responses against schistosome antigens

The antigenic complexity of *S. mansoni* stages in human hosts elicit multiple antibody responses during the course of acute and chronic human schistosomiasis. Patients who develop acute clinical disease (who are usually from non-endemic areas) clearly differ in their immune responses from those observed in most chronically infected patients from areas endemic for schistosomiasis. Comparison of immune responses in patients with acute and chronic schistosomiasis show that those with acute infection exhibit significantly higher levels of PBMC response *in vitro* to SEA, and have higher levels of IgM and IgG anti-schistosome antibodies than do individuals with chronic schistosomiasis[12 58]. Also, sera from Egyptians and Brazilians with acute schistosomiasis recognize predominantly polypeptide epitopes, primarily in adult worm extracts[56 64]. Serologically, acute and chronic schistosome infections can be distinguished on the basis of specific IgM and/or IgA titers[42 47 54] and on the basis of high IgG anti-KLH titers[4 48].

In the chronic phase, a few studies have reported some differences of antibody level and specificity between the intestinal (INT), hepatointestinal (HI) and hepatosplenic (HS) clinical groups. Cercariacidal (so-called "lethal") antibody levels are higher in patients with hepatosplenism[9 25]. Western blot analysis of sera from INT and HS patients indicate that members of both groups expressed very high levels of heterogeneity of their antibody responses, but for INT patients there is a major antigen of approximately 31 kDa that is recognized by 82% of the group and by only 13% of HS individuals. In contrast, sera from all HS patients recognized 14 kDa and 66 kDa antigens. The 14 kDa molecule was also seen by sera of 25% of INT patients but the latter was unrecognized by sera of any of the INT patients assayed[19]. Recently, it was seen that the IgG response to adult worm antigens was distinct and significantly higher in HS patients than in those in INT and HI clinical groups[64]. It has also been reported that levels of IgM (but not IgG) anti-SmW68 correlate inversely with the intensity of infection[44].

Antibody class and subclass sudies[7 41] indicate the involvement of multiple schistosome-specific and non-specific immunoglobulin isotypes in different infection conditions. High IgG4 levels are often remarkable, as are IgE levels. Isotypic regulation with the duration of infection and its possible role in resistance/susceptibility to reinfection, as well as idiotypic regulation and the relationship between the expression of dominant idiotypes on anti-SEA antibodies and morbidity are discussed in other sections.

Finally, many individuals from areas endemic for schistosomiasis also harbor multiple helminthic infections which elicit a number of cross-reactive serological responses to *S. mansoni*, and vice-versa[18]. This presents an additional complexity which should be considered in the evaluation of anti-schistosome responses in different endemic areas.

Immune response correlations with resistance to reinfection

Multiple epidemiological studies in many different endemic areas indicate that in a community the prevalence and intensity of schistome infections rise during the first decade and a half of life, and this is followed by a decline in both parameters. An immunological interpretation of these date suggests the gradual loss of current infections and the development in older individuals from endemic areas of a resistance to superinfection[11 45]. One approach to identify this resistant group was to study the intensity of re-infection after treatment among individuals of all ages whose levels of contact with water, known to contain infected

snails, was observed simultaneously. By this aproach it has been possible to distinguish an immune from a susceptible group[8]. An alternative method has been to identify uninfected individuals living in endemic areas who were stool-negative, but were known to have reasonable levels of exposure to water containing infected snails[20].

Various humoral and cellular responses have been sudied concommitantly in these different endemic populations. The relationship between intensities of re-infection following treatment of 129 children in Kenya, and various humoral responses has been studied by Butterworth and colleagues[8]. They have demonstrated high levels of IgG antibodies which can mediate eosinophil-dependent damage to schistosomula *in vitro*, and this activity was found in the sera of all children, both susceptible and resistant. Further analysis of these sera revealed a positive correlation between intensity of re-infections after treatment and the levels of IgM antibodies with specificity for a major 38 kDa schistosomular surface antigen. Furthermore, IgM-enriched fractions recognizing both schistosomular surface and egg antigens not only failed to mediate eosinophil killing of schistosomula, but also blocked the killing that was mediated by IgG fractions from the same sera. These and other date suggest that blocking antibodies, including some IgM and ineffective IgG isotypes, prevent the expression of immunity in susceptible children. A related study in Bahia (Brazil) demonstrated an association of resistance after chemotherapy with seroreactivity to a 37 kDa larval surface antigen[22].

Another antibody response against a specific epitope that may distinguish between resistant/ susceptible individuals was reported in the study of infected and non-infected individuals in another endemic area in Brazil, Divino do Traira[20]. It was shown that many of the subjects in the stool-negative, non-infected group were seropositive to a soluble adult worm preparation (SWAP), despite their having no record of previous infection or treatment. The individuals in this non-infected (endemic normal) group, while indistinguishable from infected subjects in both SWAP and glutathione-S-transferase (GST) responses, had significantly elevated antibody levels to paramyosin. In addition, in infeced/treated individuals anti-paramyosin antibody levels increased after treatment and remanined elevated only in those displaying complete parasitological cure.

A recent study suggests that significant degree of protective immunity against *S.haematobium* infection also gradually develops in a community in the Gambia[38]. Analysis of sera of these patients indicates that the IgE antibody levels (an isotype which participates in several reported *in vitro* anti-parasite effector mechanisms) were higher in adults, and the levels of IgG4 antibodies against worm an egg antigens (a potential blocking isotype) were highest in the 10-14 year-old group. Re-infection studies in this community have supported the hypothesis that the gradual development of immunity to infection may in part depend on pathways involving IgE/IgG4 rations, with the IgE mediating (or correlating with) resistance, and high levels of IgG4 antibodies blocking these mechanisms in younger patients.

PBMC responses to different antigen preparations have also been associated with the presence or absence of re-infection after treatment. In a study of re-infection after treatment in 18 Egyptian patients, those who became re-infected were similar to those who did not, in terms of their pretreatment intensities of infection or locations of their households in relationship to water contact sites. However, they did differ in their most recent PBMC responses, prior to their re-infections. Those patients who failed to become re-infected showed increased responses to SEA, CAP and SWAP antigens after treatment and these levels remained high throughout the study period[13]. A possibly related observation is the finding of high PBMC responses to SWAP, CERC, and especially SEA, of essentially all putatively resistant, endemic normals living in the three consecutively studied endemic areas of Divino do Traira, Siqueira and Bernardo (Brazil) (G Gazzinelli: work in progress). This is in shap contrast to most of those in the infected group, who generally present a much lower response to SEA[13 14 15].

Immune response correlations with morbidity

Perhaps the most studied aspect of human immune responses during schistosomiasis are comparisons between the responsiveness of PBMC of patients with INT vs. HS infections[12 15 26 53].

Based on studies in many different endemic areas, the general interpretation of such studies is that although worm burden is seen to correlate with severity of disease in autopsy studies[10], this correlation is not absolute, and the intensity of a patient's immune response to certain antigens of the parasite may contribute to the overall outcome of that a patient's disease. Multiple differences occur between the immune capabilities of INT and HS patients, but an important consideration is the extent of the patient's disease. In geral, studies on hospitalized HS patients (who are often decompensated and hospitalized for splenectomy and hemodynamic surgery for hypertension) discover patients in an anergic state, with altered T cell subset populations[16] and very low-to-non-responsive immune systems[15]. However, a very different picture is observed when field cases of ambulatory HS patients are studied[15 26]. Here on usually finds that these HS patients are hyper-responsive to schistosomal antigens, especially SEA, and especially in contrast to those (with apparently well regulated immune response) INT patients[15 65]. Recently, it has been seen that severe infection is associated with a higher frequence of demonstrable responses to fractionated SWAP by T cell Western assays[5]. Also, HS patients PBMC respond to T cell Western antigen fractions in the lower molecular weight range (<21 kDa) than do those of INT patients. As with Ellner et al[26] and Hafez, et al[37] in schistosomiasis mansoni patients, in shistosomiasis japonica, Ohta et al[55] noted fewer low responders to adult worm extracts in an HS group than asymptomatic patients.

A number of other immunological parameters have been correlated with disease severity, such as the occurrence of circulating IC in patients with HS infections[30], higher levels of anti-worm glycoprotein, but not anti-egg, antigens in HS patients[64], and the detection of lower INF-gamma production by HS individuals[67].

The immunogenetics of schistosomiasis has been investigated by several groups [1 2 37 40 55 63]. Various different HLA haplotypes have been associated with hepatosplenism, and it appears that some patients have an immunogenetic predisposition to severe disease. There is also evidence that host genetics (albeit not yet immunogenetics) could play a role in human resistance/susceptibility issues[3].

RESUMO

O estudo da resposta imune de pacientes com esquistossomose progride em função do avanço tecnológico, apesar das dificuldades de manipulação in vivo *deste complexo sistema parasita-hospedeiro. A ênfase nos estudos tem sido mais freqüentemente dirigida para comparações entre grupos característicos de indivíduos, tais como infectado/não infectado, reinfectado/não reinfectado, assintomático/hepato-esplênico, com intensidade de infecção alta/baixa, etc. Baseado nessas comparações tem-se obtido informações que permitem conjecturas razoáveis com relação à regulação imunológica, susceptibilidade e resistência e até mesmo considerações sôbre mecanismos, que têm sido mais apropriadamente analisados, entretanto, em sistemas experimentais. Tais estudos têm como finalidade a compreensão das causas e eventos que levam à morbidade e ao desenvolvimento de uma vacina humana anti-esquistosoma.*

Palavras-chaves: Esquistosomíase. Resposta imune. Imuno-regulação.

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